

*Full Length Research Paper*

# Antifatigue effect of aqueous extract of salvia in endurance training rats' skeletal muscle

Shao JiPing

Department of Physical education, Lanzhou Commercial College, Lanzhou, 730020, PR China.  
E-mail: 857015283@qq.com. Tel:+8613893225716

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**This study evaluated the anti-oxidant potential of salvia extracts in enhance training rats. The salvia extracts were administered to endurance exercise rats by gavage for 28 days. In addition to antioxidant enzymes activities evaluation, several functional enzymes assays (e.g., LDH, CK and CHE activities) were employed to examine the effects of salvia extracts on the oxidative injury in endurance exercise rats. The results from this study demonstrated that salvia extracts treatment significantly reduced lipid peroxidation, LDH, CK activities levels, enhanced antioxidant enzymes, and CHE activities in the skeletal muscle of endurance exercise rats. Taken together, the salvia extracts may decrease oxidative injury induced by endurance training and display strong antifatigue effect.**

**Key words:** Salvia, malondialdehyde, superoxide dismutase, endurance training.

## INTRODUCTION

Oxidative stress may occur due to an increase in free radical production and/or a decrease in antioxidant defenses. In muscle, mitochondria are one source of reactive species that include superoxide ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ), and possibly hydroxyl radical ( $HO\cdot$ ) (Boveris and Chance, 1973; Boveris et al., 1976; Chance et al., 1979; Barja, 1999). The recent discovery that mitochondria generate nitric oxide ( $NO\cdot$ ) also has implications for oxidant production and mitochondrial function (Giulivi et al., 1998) during exercise. In moderate amounts, nitric oxide may regulate respiration (Giulivi et al., 1998), but it may also react with radicals such as  $O_2^{\cdot-}$  to form peroxynitrite ( $ONOO^-$ ), a powerful oxidant (Ischiropoulos et al., 1992). Lipid peroxidation is a complex process and the cell membranes enriched with PUFAs are more prone to lipid peroxidation, resulting in the loss of their fluidity and permeability properties leading to tissue damage in old subjects.

A limited number of endurance training studies has been carried out in SID rats to determine whether training

would prevent the progressive decline in cardiac function (Paulson et al., 1987) or alter the responses of myocardium to ischemia (Riggs et al., 1992). To our knowledge, there is no report on the effects of salvia extract on oxidative stress in skeletal muscle of the endurance training rats. In this study, we examine effect of salvia extract on oxidative injury induced by endurance training.

## MATERIALS AND METHODS

Aqueous extract from salvia was prepared in our laboratory. Content of tanshinone in the extract was 92.6%.

### Animals

The China Institutional Animal Care and Use Committee approved the experimental procedures. Fifty male Sprague-Dawley rats (Charles River, Wilmington, MA) were used in this study. They were housed in a light (12 h light:dark cycle) and temperature (20 to 22°C) controlled animal facility. Food and water were available *ad libitum*. In order to determine the effects of salvia extract on oxidative injury in endurance training rats, animals were randomly divided into five groups: control sedentary (CS), endurance exercise model control (EEMC), and low (100 mg/kg B.W.), middle (200 mg/kg B.W.) and high (300 mg/kg B.W.) doses of salvia extract-treatment groups (SE).

**Abbreviations:** ChE, cholinesterase; CK, creatine kinase; LDH, lactate dehydrogenase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

**Table 1.** Effect of Salvia extract on MDA level in rats' skeletal muscle.

Group	MDA (nmol/mg)
CS	8.62±0.93
EEMC	14.51±1.42 <sup>b</sup>
SE (I)	12.05±1.06 <sup>c</sup>
SE (II)	10.41±0.88 <sup>d</sup>
SE (III)	8.29±0.58 <sup>d</sup>

<sup>b</sup> P<0.01, vs normal rats; <sup>c</sup>P<0.05, <sup>d</sup> P<0.01, vs model rats, (CS), endurance exercise model control (EEMC), and low, middle and high salvia extract-treatment groups (SE).

### Exercise program

Rats in EEMC group received endurance training. Rats in SE groups received endurance training and fed with salvia extract. The exercise program consisted of a four-week endurance training period followed by a four-week period of high-intensity exercise. After being familiarized with a motor-driven rodent treadmill for 2 days, animals ran on a treadmill at 10 m/min, 0% slope for 10 min/day during the first week. The intensity and duration of the exercise training were progressively increased until at week 4 the animals were running at 25 m/min, 0% slope for 40 min/day. During the 5 to 8 week period, exercise capacity was evaluated by beginning with a treadmill speed of 15 m/min, and increasing it 5 m/min every 20 min until a speed of 30 m/min was reached (10° incline throughout). Rats were run until they could no longer maintain the pace of the treadmill, that is until they were completely exhausted, defined as the inability to continue running despite contact with a shock bar located at the rear of the treadmill belt (Bedford et al., 1979; Liu et al., 2005; Pan, 2008; Yatabe et al., 2003). The animals were sacrificed 24 h after the last exercise session. The skeletal muscle was dissected out, rinsed in PBS and used immediately for MDA level and SOD activity.

### Biochemical analysis

#### Determination of MDA

Malondialdehyde (MDA) levels, an indicator of free radical generation that increases at the end of the lipid peroxidation, were estimated by the double heating method of Draper and Hadley (1990). The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. The level of MDA is expressed as nanomoles per gram protein.

#### Determination of SOD

SOD activity was measured using nitroblue tetrazolium (NBT) reduction assay following the reduction of nitrite by a xanthine-xanthine oxidase system, which is a superoxide anion generator. One unit of SOD is defined as the amount that shows 50% inhibition.

#### Measurement of GSH-Px activity levels

GSH-Px activity levels were determined by the method of Paglia and Valentine.17. The concentrations of reagents in the assay

mixture prepared fresh daily were as follow: 150 mM potassium phosphate buffer, 5 mM EDTA, 0.5 mM sodium azide, 2 mM reduced glutathione, 0.24 mM NADPH and 1U ml<sup>-1</sup> glutathione reductase. Results are expressed as Umg<sup>-1</sup> protein.

#### Measurement of LDH activity

LDH activity was determined working in the oxidative direction, that is using L-lactate (Acros Organics, 189870010) as substrate, thus producing pyruvate, and thereby reducing NAD<sup>+</sup> to NADH+H<sup>+</sup>. A process was further coupled to the primary reaction, in that the non-fluorescent resazurin (Sigma, R-2127) was used as an intermediate and reduced quantitatively by NADH+H<sup>+</sup> producing the highly fluorescent substance resorufin. The enzyme diaphorase was used to mediate the latter step of oxidation/reduction.

#### Measurement of CK and CHE activities

CK activity was analyzed with Wiener lab kits run in an automatic analyzer (Autolab Boehringer). The enzymatic plasma CK activity was measured at 37°C. The internal quality control for the analyte was performed in parallel with the tests by measuring the levels of the commercial serum control. Cholinesterase activity is measured colorimetrically.

#### Statistical analysis

Values were presented as mean±SD. Statistical analysis was done using the SPSS ver. 17.0 (SPSS Inc., Chicago, IL) by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls post hoc test. P value < 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

ROS may modulate the antioxidant enzyme activities by regulating the mRNA levels through activation of signaling pathways (Fulle et al., 2004). According to Franco et al. (1999), the induction of antioxidant enzyme mRNA levels coincide with increases in oxidative damage of proteins, supporting the postulated relationship between oxidative stress and antioxidant enzyme mRNA expression. In fact, ROS play a very important role to regulate several cell functions, acting as second messengers, and activating specific redox-sensitive transcription factors, such as AP-1 and NF-kB (Dalton et al., 1999; Zhou et al., 2001; Khassaf et al., 2003).

Lipid peroxidation is a process through which reactive oxygen species and free radicals break down lipid molecules. Lipids are a major component of the cell membrane. Thus, ROS and free radicals can break down cell membranes through lipid peroxidation, leading to cell death (Henderson et al., 2006). MDA is an indicator of lipid peroxidation processes which involve the formation of free radical species (Nielsen et al., 1997).

Results are shown in Table 1. MDA was regarded as main parameters that reflected the degree of oxidative injury in rats' skeletal muscle. Compared with normal group, the parameter was significantly increased in model group (P<0.01). Salvia extract (100, 200 and 300 mg·kg<sup>-1</sup>)

**Table 2.** Effect of salvia extract on SOD and GSH-Px levels in rats' skeletal muscle.

Group	SOD	GSH-Px
CS	216.3±17.5	32.09±1.98
EEMC	143.8±11.9 <sup>b</sup>	20.11±1.74 <sup>b</sup>
SE (I)	173.9±20.7 <sup>d</sup>	22.87±2.54 <sup>d</sup>
SE (II)	190.5±16.2 <sup>d</sup>	27.67±2.22 <sup>d</sup>
SE (III)	208.4±19.3 <sup>d</sup>	31.36±2.73 <sup>d</sup>

<sup>b</sup> P<0.01, vs normal rats; <sup>d</sup> P<0.01, vs model rats.

**Table 3.** Effect of Salvia extract on LDH activities in rats' skeletal muscle.

Group	LDH
CS	1316.4±141.6
EEMC	1951.9±152.7 <sup>b</sup>
SE (I)	1746.2±136.5 <sup>d</sup>
SE (II)	1599.5±163.1 <sup>d</sup>
SE (III)	1501.3.4±142.6 <sup>d</sup>

<sup>b</sup> P<0.01, vs normal rats; <sup>d</sup> P<0.01, vs model rats.

**Table 4.** Effect of Salvia extract on CK and CHE activities in rats' skeletal muscle.

Group	CK (U/mg)	CHE (U/mg)
CS	1.99±	7.34±0.53
EEMC	3.15±0.52 <sup>b</sup>	3.17±0.28 <sup>b</sup>
SE (I)	2.78±0.35 <sup>d</sup>	4.92±0.35 <sup>d</sup>
SE (II)	2.41±0.22 <sup>d</sup>	5.27±0.41 <sup>d</sup>
SE (III)	2.26±0.19 <sup>d</sup>	6.73±0.36 <sup>d</sup>

<sup>b</sup> P<0.01, vs normal rats; <sup>d</sup> P<0.01, vs model rats.

could remarkably decrease these elevated parameters (P<0.05-0.01).

SOD plays a key role in detoxifying superoxide anions, which otherwise damages the cell membranes and macromolecules. SOD specifically scavenges the superoxide radicals by catalyzing their dismutation to hydrogen peroxide and oxygen. Hydrogen peroxide has been proposed to be the most toxic ROS for human spermatozoa (Alvarez et al., 1987; Aitken et al., 1993). Catalase enhances the degradation of hydrogen peroxide to oxygen and water. Treatment with SOD and catalase reduced the degree of testicular damage in experimental acute torsion (Henry and Turner, 1996). In endurance exercise rats, the activities of SOD and GSH-Px were significantly decreased in rats' skeletal muscle (Table 2) (P<0.01). The endurance exercise rats treated with salvia extract exhibited a significant increase in the activities of SOD and GSH-Px in a dose-dependent manner (P<0.01).

This indicated that salvia extract may enhance SOD and GSH-Px activity in skeletal muscle in endurance exercise rats.

Lactate dehydrogenase, also called lactic dehydrogenase, or LDH, is an enzyme found in the cells of many body tissues, including the heart, liver, kidneys, skeletal muscle, brain, red blood cells, and lungs. It is responsible for converting muscle lactic acid into pyruvic acid, an essential step in producing cellular energy (Dieli-Conwright et al., 2009). In endurance exercise rats, the activities of LDH were significantly increased in rats' skeletal muscle (Table 3) (P<0.01). The endurance exercise rats treated with salvia extract exhibited a significant increase in the activities of LDH in a dose-dependent manner (P<0.01).

Creatine kinase plays a key role in the energy metabolism of heart and skeletal muscle cells (Saks et al., 1975; Seraydarian and Abbott, 1976). Because the enzyme leaks into the blood during reperfusion after ischaemia, levels of creatine kinase activity in serum are commonly used in clinical pathology to assess the extent of myocardial infarcts (see, for example, De Leiris and Hearse, 1986). The creatine kinase (CK) isoenzymes catalyse the synthesis of phosphocreatine (PCr) and its subsequent use in the regeneration of ATP in cell types where the consumption of ATP is rapid and or sudden.

Table 4 shows the effect of salvia extract on CK and CHE activities in rats' skeletal muscle. The CK and CHE activities in rats' skeletal muscle increased or decreased, along with the increase of the endurance training. Salvia extracts exhibit different antifatigue effect by inhibiting the CK and reducing CHE activities.

### Conclusion

Our study provided the first report on the antioxidant and immunomodulatory effects of salvia extract in endurance training rats. Significant changes in the activities of antioxidant enzymes, LDH and CK were identified in skeletal muscle. Salvia extract displays considerable potency in anti-inflammatory, antioxidant activity and has prominent antifatigue effects on endurance training rats. Future studies will provide deeper insight into the anti-fatigue activity of salvia extract.

### REFERENCES

- Aitken RJ, Buckingham DW, Harkiss D (1993). Use of a xanthine oxidase free radical generating system to investigate the cytotoxic effects of reactive oxygen species on human spermatozoa. *J. Reprod. Fertil.*, 97: 441-450.
- Alvarez JG, Touchstone JC, Blasco L, Storey BT (1987). Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. *J. Androl.*, 8: 338-348.
- Barja G (1999). Mitochondrial free radical generation: Sites of production in states 4 and 3, organ specificity and relationship with aging rate. *J. Bioenerg. Biomembr.*, 31(4): 347-366.
- Bedford TG, Tipton CM, Wilson NC, Oppliger RA, Gisolfi CV (1979).

- Maximum oxygen consumption of rats and its changes with various experimental procedures. *J. Appl. Physiol.*, 47: 1278–1283.
- Boveris A, Cadenas E, Stoppani AO (1976). Role of ubiquinone in the mitochondrial generation of hydrogen peroxide, *J. Biochem.*, 156: 435-444.
- Boveris A, Chance B (1973). The mitochondrial generation of hydrogen peroxide, *Biochem. J.*, 134: 707-716.
- Draper HH, Hadley M (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods. Enzymol.*, 186: 421-431.
- Chance B, Sies H, Boveris A (1979). Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.*, 59: 527-605.
- Dalton TP, Shertzer HG, Puga A (1999). Regulation of gene expression by reactive oxygen. *Annu. Rev. Pharmacol. Toxicol.*, 39: 67–101.
- De Leiris J, Hearse DI (1986). Myocardial enzyme leakage as an indicator of cellular injury: Principles and application. In *Methods in Studying Cardiac Membranes*, Vol. f (edited by DHALLA, N. S.) Florida: CRC Press, pp. 253-277.
- Dieli-Conwright CM, Spektor TM, Rice JC, Todd Schroeder E (2009). Hormone therapy attenuates exercise-induced skeletal muscle damage in postmenopausal women. *J. Appl. Physiol.*, 107: 853–858.
- Franco AA, Odom RS, Rando TA (1999). Regulation of antioxidant enzyme gene expression in response to oxidative stress and during differentiation of mouse skeletal muscle. *Free Radic. Biol. Med.*, 27: 1122–1132.
- Fulle S, Protasi F, Di Tano G, Pietrangelo T, Beltramin A, Boncompagni S, Vecchiet L, Fano G (2004). The contribution of reactive oxygen species to sarcopenia and muscle ageing. *Exp. Gerontol.*, 39: 17–24.
- Giulivi C, Poderoso JJ, Boveris A (1998). Production of nitric oxide by mitochondria. *J. Biol. Chem.*, 273: 11038-11043.
- Henderson D, Bielefeld EC, Harris KC, Hu BH (2006). The role of oxidative stress in noise-induced hearing loss. *Ear Hear.*, 27: 1-19.
- Henry MP, Turner TT (1996). Rescue of testicular function following acute experimental torsion. *Proc. AUA.*, 155: 442A.
- Ischiropoulos H, Zhu L, Beckman JS (1992). Peroxynitrite formation from macrophage-derived nitric oxide. *Arch. Biochem. Biophys.*, 298(2): 446-451.
- Khassaf M, McArdle A, Esanu C, Vasilaki A, McArdle F, Griffiths RD, Brodie DA, Jackson MJ (2003). Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. *J. Physiol.*, 549: 645–652.
- Liu CC, Huang CC, Lin WT, Hsieh CC, Huang SY, Lin SJ, Yang SC (2005). Lycopene supplementation attenuated xanthine oxidase and myeloperoxidase activities in skeletal muscle tissues of rats after exhaustive exercise. *Br. J. Nutr.*, 94: 595–601.
- Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P (1997). Plasma malondialdehyde as biomarker for oxidative stress: Reference interval and effects of life-style factors. *Clin. Chem.*, 43: 1209-1214.
- Pan SS (2008). Alterations of atrial natriuretic peptide in cardiomyocytes and plasma of rats after different intensity exercise. *Scand. J. Med. Sci. Sports*, 18: 346–353.
- Paglia DE, Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, 70: 158–168.
- Paulson DJ, Kopp SJ, Peace DG, Tow JP (1987). Myocardial adaptation to endurance exercise training in diabetic rats. *Am. J. Physiol.*, 252: R1073-1081.
- Riggs CE, Collins G, Taylor M (1992) Streptozotocin-induced diabetes and the effects of endurance exercise training. *Diabetes Res.*, 19: 177-185.
- Saks VA, Chernousova GB, Gukovsky DE, Smirnov VNC, AZOV EI (1975). Studies on energy in heart cells. *Eur. J. Biochem.*, 57: 273-290.
- Seraydarian MW, Abbot BC (1976). The role of the creatine-phosphorylcreatine system in muscle. *J. Mol. Cell Cardiol.*, 8: 741-746.
- Yatabe Y, Miyakawa S, Miyazaki T, Matsuzaki Y, Ochiai N. (2003). Effects of taurine administration in rat skeletal muscles on exercise. *J. Orthop. Sci.*, 8: 415–419.
- Zhou LZ, Johnson AP, Rando TA (2001). NF kappa B and AP-1 mediate transcriptional responses to oxidative stress in skeletal muscle cells. *Free Radic. Biol. Med.*, 31: 1405–1416.